EVALUATION OF THE PHOTOPROTECTIVE ROLE OF QUERCETIN TO SELECTED LIGHT-ADAPTED AND SHADE-TOLERANT PLANT SPECIES

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DEDICATION

The thesis is dedicated to my parents Alhaji Idris Yusuf and Hajiya Hauwa Muhammad Babayo for their prayers, advice and encouragement, which guided me to this achievement. The thesis is also dedicated to my husband Zakiyyu, my siblings, and my children Muhammad, Iman, Hauwa, and Muhammad. May Allah bless them.

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ABSTRACT

Plants respond to different light intensities depending on their genetic make-up, mutation and other environmental conditions. *Mikania micrantha, Clidemia hirta,* and *Tetracera sarmentosa* were selected so as to analyse how varying light intensity affects some gas exchange characteristics, pigment and production of total flavonoid content. Besides, the study was aimed at evaluating the photoprotective role of quercetin to the selected plants, in addition to correlating how the production of flavonoids affects the plants photosynthesis. The photosynthetic rates of the selected plants were determined using LI-6400. The chlorophyll, carotenoid, anthocyanin, flavonoid, antioxidant enzymes, malondialdehyde, soluble sugar and soluble protein contents were quantified using spectroscopic techniques. Quercetin was quantified using high-performance liquid chromatography (HPLC). Sun-exposed plants were having the maximum photosynthesis and quercetin content compared with semi-shaded or fully shaded plants. The highest quercetin content was recorded for sun-exposed *C. hirta* (0.950 ± 0.023 μg/ml) while the lowest was recorded for shaded *T. sarmentosa* (0.13 ± 0.007 μg/ml). The highest oxidative stress was recorded for sun-exposed *T. sarmentosa* (6.19 ± 019 μg/ml) which was also having the lowest quantum efficiency of photosystem II (0.509 ± 0.003). Superoxide dismutase activity was lowest under sun-exposed *C. hirta* (1.86 ± 0.06 U/mg protein), while peroxidase and catalase were lowest under Sun-exposed *T. sarmentosa* (59.59 ± 2.67 and 3.75 ± 0.17) U/mg protein respectively. The result obtained makes it possible to accept the generated hypothesis of the study because the quercetin content was higher when the antioxidant enzymes of the plants were low. This leads to a conclusion that increase in the production of secondary metabolites at high light intensity is not due to high CO_2 assimilation rate, but rather due to the production of photoprotective metabolites to conquer the light stress.

ABSTRAK

Tindak balas terhadap keamatan cahaya yang berlainan bergantung kepada genetik, mutasi dan keadaan alam sekitar yang lain. *Mikania micrantha, Clidemia hirta,* dan *Tetracera sarmentosa* dipilih untuk menganalisa bagaimana variasi keamatan cahaya mempengaruhi beberapa ciri pertukaran gas, pigmen dan pengeluaran jumlah kandungan flavonoid. Selain itu, penyelidikan ini bertujuan untuk menilai peranan fotoprotektif kuercetin terhadap tanaman pilihan, di samping mengaitkan bagaimana pengeluaran flavonoid mempengaruhi fotosintesis tumbuhan. Fotosintesis tumbuhan pilihan ditentukan menggunakan LI-6400. Klorofil, karotenoid, antosianin, flavonoid, enzim antioksidan, malondialdehid, gula larut dan kandungan protein larut dikira secaro teknik spektroskopi. Kuercetin dikira menggunakan kromatografi cecair prestasi tinggi (HPLC). Tumbuhan yang terdedah kepada matahari mempunyai kandungan fotosintesis dan kuercetin yang maksimum berbanding dengan tumbuh-tumbuhan teduh separuh atau teduh penuh. Kandungan kuercetin tertinggi direkodkan oleh *C. hirta* (0.950 ± 0.023 μg/ml) manakala yang paling rendah direkodkan oleh *T. sarmentosa* (0.13 ± 0.007 μg/ml). Tekanan oksidatif tertinggi direkodkan oleh *T. sarmentosa* (6.19 ± 019 μg / ml) yang terdedah kepada matahari yang juga mempunyai kecekapan terendah dalam fotosistem II (0.509 ± 0.003). Aktiviti superoxide dismutase adalah terendah pada *C. hirta* (1.86 ± 0.06 U / mg protein) manakala peroksidase dan catalase adalah terendah pada *T. sarmentosa* (59.59 ± 2.67 dan 3.75 ± 0.17) U / mg protein masing-masing. Keputusan yang diperoleh memungkinkan untuk menerima hipotesis kajian kerana kandungan kuercetin lebih tinggi apabila enzim antioksidan tumbuhan adalah rendah. Secara kesimpulannya, peningkatan pengeluaran metabolit sekunder semasa keamatan cahaya yang tinggi tidak disebabkan oleh kadar asimilasi CO_2 yang tinggi, tetapi disebabkan oleh pengeluaran metabolit pelindung cahaya untuk menangani tekanan cahaya.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	-	Percent
^{o}C	-	Degree Celsius
θ	-	Curve curvature
Ø	-	Apparent quantum yield
ϕPSII	-	Efficiency of PSII photochemistry
µg/ml	-	Microgram per milliliter
µl	-	Microlitre
µm	-	Micrometre
A/C	-	Photosynthetic CO_2 response curve
A/Q	-	Photosynthetic light response curve
$AlCl_3$	-	Aluminium chloride
A_{max}	-	Maximum net assimilation rate
A_{net}	-	Net assimilation rate
ANOVA	-	Analysis of variance
ATP	-	Adenosine triphosphate
C_{ar}	-	Carotenoid
CAT	-	Catalase
Ci	-	Intercellular CO_2
CO_2	-	Carbon dioxide
Cp	-	Crude protein
Df	-	Dilution factor
DNA	-	Deoxyribonucleic acid
Dw	-	Dry weight
EDTA-Na_2	-	Disodium ethylenediaminetetraacetate dehydrate
ETR	-	Electron transport rate
Fm	-	Maximal fluorescence
Fm^{l}	-	Light adapted maximal fluorescence

Fo	-	Minimal fluorescence
Fo^{l}	-	Light adapted minimal fluorescence
Fs	-	Steady state fluorescence
Ft	-	Feet
Fv/Fm	-	Efficiency of photosynthesis
Fw	-	Fresh weight
g	-	Gram
H_2O	-	Water
H_2O_2	-	Hydrogen peroxide
H_2SO_4	-	Sulfuric acid
H_3PO_4	-	Phosphoric acid
HPLC	-	High-performance liquid chromatography
IRGA	-	Infrared gas analyser
J_{max}	-	Maximum electron transport rate
LCP	-	Light compensation point
LED	-	Light-emitting diode
LSP	-	Light saturation point
m	-	Meter
M	-	Molar
MDA	-	Malondialdehyde
ml	-	Milliliter
mM	-	Millimolar
MW	-	Molecular weight
NBT	-	Nitro blue tetrazolium
nm	-	Nanometer
NPQ	-	Non-photochemical quenching
O_2	-	Oxygen
PAL	-	Phenylalanine ammonia lyase
PAR	-	Photosynthetic active radiation
pH	-	$- \log [H^+]$
PHOT	-	Phototropin photoreceptor
PPFD	-	Photosynthetic photon flux density
PSI	-	Photosystem I
PSII	-	Photosystem II

PTNJ	-	Perbadanan Taman Negara Johor
qp	-	Photochemical quenching
Rd	-	Dark respiration
ROS	-	Reactive oxygen species
RuBisCO	-	Ribulose-1, 5-bisphosphate carboxylase
SD	-	Standard deviation
SLA	-	Specific leaf area
SOD	-	Superoxide dismutase
SPSS	-	Statistical Package for the Social Science
TBA	-	Thiobarbituric acid
TCA	-	Trichloroacetic acid
TFC	-	Total flavonoid content
TSS	-	Total soluble sugar
UTHM	-	Universiti Tun Hussein Onn Malaysia
UV	-	Ultraviolet
UV-R8	-	UV-Resistance Locus 8
V_{cmax}	-	Maximum carboxylation rate of RuBisCO
W / V	-	Weight / Volume
YPF	-	Yield photon flux

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CHAPTER 1

INTRODUCTION

1.1 Background and rationale of the study

The environment does not have a constant stable condition, it always changes, and these changes can lead to various effects in the structural, biochemical and gas exchange characteristics of a leaf including its shape, curling degree, and its surface characteristics. One of the most important environmental factor affecting plants is light. Light affects the growth and development of plants by influencing the morphology of a single leaf as well as the morphology of the whole plant. Plants can adapt to different light intensity depending on their environment. This adaptation would be possible if plant changes the distribution of its biomass and its morphology, in order to be able to utilize the amount of light they receive, so as to survive (Xue *et al.*, 2011).

Changes in the amount of light intensity received by a plant can affect the gas exchange characteristics. There is no fixed value or range of values that indicate the difference in photosynthesis of sun-exposed and shaded plants. This is because each plant responds to light differently. Also, some plants can have higher photosynthesis under higher light intensity (greater than 1000 μmol $m^{-2}s^{-1}$) while others have higher photosynthesis under lower light intensity (less than 1000 μmol $m^{-2}s^{-1}$). The compensation and saturation points of sun-exposed leaves exceeds that of shaded leaves (DeWalt, Denslow, & Hamrick, 2004; Lambers, Chapin, & Pons, 2008; Li *et al*., 2016; Liu *et al*., 2013; Qin *et al*., 2012; Xue *et al*., 2011), but in some plants like *Aeschynanthus longicaulus* (Li *et al*., 2014), *Tetrastigma hemsleyanum* (Dai *et al*., 2009), and *Lindera melissifolia* (Aleric & Kirkman, 2005), higher compensation and

saturation points were recorded at lower light intensity. Moreover, photosynthetic carboxylation capacity, electron transport capacity, quantum yield and curvature of light response curve varies depending on the level of light intensity received by a plant (Li *et al*., 2017; Slattery *et al*., 2017; Vialet-Chabrand *et al*., 2017). For instance, the maximum photosynthesis (A_{max}) of *Chromera velia* was higher when the plant was grown at 406 ±30 µmol O_2 mg Chl^{-1} h^{-1} compared to when grown at 239 ±60 µmol O_2 mg Chl^{-1} h^{-1} (Belgio *et al*., 2017) while *Changium smyrnioides* (Wang *et al*., 2017) requires a semi-shaded environment for its maximum photosynthesis. Recent studies on *Eucheuma denticulatum* (Borlongan *et al*., 2017), *Batrachospermum turfosum* (Aigner *et al*., 2017), *Kappaphycus* sp (Borlongan *et al*., 2017), *Typha latifola* (Jespersen, Brix, & Sorrell, 2017), *Laminaria digitate* (Migné *et al*., 2018), *Triticum aestivum* (Chen *et al*., 2016), *Lactuca sativa* (Fu *et al*., 2017), and *Vitis vinifera* (Li *et al*., 2017) indicated that the level of light intensity received by a plant can affect the plant photosynthesis. The photosynthesis of *Oryza sativa* was higher under high light intensity (Gu *et al*., 2017). Other plants with higher photosynthesis under high light intensity include *Brassica campestris* (Zhu *et al*., 2017), *Betula pendula* (Niglas *et al*., 2017), and *Phaseolus vulgaris* (Gauthier *et al*., 2018).

Besides, the quality of light has an impact on photosynthesis (Dou *et al*., 2017). For example, the photosynthesis rates, stomatal conductance and transpiration rates of *Dianthus caryophyllus* grown under blue light emitting diode (LED) was higher (7.2 µmol CO_2 $m^{-2}.s^{-1}$) than that grown under red LED (4.1 µmol CO_2 $m^{-2}.s^{-1}$) (Manivannan *et al*., 2017). Likewise, the photosynthesis of *Solanum lycopersicum* increase significantly when the plant was grown under a combination of red and blue LED light (Li *et al*., 2017). Other recent studies on effect of light quality on photosynthesis include that of *Populus trichocarpa* (Momayyezi & Guy, 2017), *Synechocystis* sp. (Oshita, Suzuki, & Kawono, 2018), *Cucumis sativus* (Särkkä *et al*., 2017), *Cyclocarya paliurus* (Yang *et al*., 2017), *Camptotheca acuminuta* (Yu *et al*., 2016) and *Lactuca sativa* (Zhen & van Iersel, 2017). Species cultivar (Slattery *et al*., 2017), nutrient availability (Özer, 2017) and temperature (Lu *et al*., 2017; Wu *et al*., 2018) can influence the effect of light on plant photosynthesis.

Apart from variation in photosynthesis, shaded leaves differ from sun-exposed leaves in their structure and biochemical characteristics. The stomatal density (Jespersen *et al*., 2017; Manivannan *et al*., 2017; Sakhonwasee, Tummachai,

& Nimnoy, 2017; Sevik *et al*.,2017; Zheng & Van Labeke, 2017), fresh and dry weight (Pedroso *et al*., 2017, Rehana *et al*., 2018), leaf area (Silva *et al*., 2017), and leaf thickness (Vialet-Chabrand *et al*., 2017) differs among shaded and sun-exposed species.

In a shaded environment, the light received is weak and therefore the plant will produce more photosynthetic pigments (Belgio *et al*., 2017; Chuyong & Acidri, 2017; Fu *et al*., 2017; Jespersen *et al*., 2017; Silva *et al*., 2017; Wang *et al*., 2017, Zhu *et al*., 2017). Light quality can also influence the photosynthesis pigments of a plant (Rabara *et al*., 2017; Singh *et al*., 2017; Yu *et al*., 2016; Zheng & Van Labeke, 2017) thereby affecting the chlorophyll *a*/B ratio.

Light intensity can affect the production of secondary metabolites like quercetin. Quercetin is a yellow flavonol which plays an important role in protecting plants against stress. It acts as an antioxidant molecule and UV-filter (Agati *et al*., 2013; Brunetti *et al*., 2013). It can protect plants against microorganisms; for example, *Vitis vinifera* accumulates quercetin for protection against *Plasmopara viticola* (Ali *et al*., 2012). Moreover, quercetin plays a vital role in the interaction of a plant with its environment (Mierziak, Kostyn, & Kulma, 2014).

The total flavonoid content of a plant can be affected depending on the light intensity. For instance, *Glycine max* seedling (Yuan *et al*., 2015) produces a high amount of flavonoids under high light intensity. *Lithocarpus litseifolius* (Li *et al*., 2016) and *Zingiber officinale* (Ghasemzadeh *et al*., 2010) accumulate high amount of flavonoid under lower light intensity (60 % of sunlight for the former and 310 μmol $m^{-2}.s^{-1}$ for the latter). *Labisia pumila* accumulates more flavonoid when grown at 630 μmol $m^{-2}.s^{-1}$ (Karimi *et al*., 2013). *Anoectochilus roxburghii* produced a high amount of flavonoid under blue light (Ye *et al*., 2017).

Other studies on the effect of light intensity and quality on accumulation of flavonoids include that of *Hyptis marrubioides* (Pedroso *et al*., 2017), *Anacardium othonianum* (Gazolla *et al*., 2017), *Chlorella miniata* (Sozmen *et al*., 2018), *Berberis microphylla* (Arena, Postemsky, & Curvetto, 2017), *Lactuca sativa* (Bian *et al*., 2018; Liu *et al*., 2018; Pérez-López *et al*., 2018), *Cannabis sativa* (Gianmaria, Gianpaolo, & Stiina, 2018), *Anoectochilus roxburghii* (Chen *et al*., 2017), *Cyclocarya paliorus* (Liu *et al*., 2018), *Abelmoschus esculentus* (Irshad *et al*., 2018), *Elephantopus scaber* (Dawiyah *et al*., 2018) and *Fagopyrum esculentum* (Nam *et al*., 2018).

The variation in flavonoids accumulation among plant species can be due to the complex metabolism of flavonoids. In heliophytes, the activity of antioxidant enzymes decreases under lower light intensity (Li *et al*., 2016), which increase reactive oxygen species (ROS). Due to an increase in ROS, more flavonoids would be synthesized in order to scavenge the ROS and protect the plant (Li *et al*., 2016). The activity of antioxidant enzymes of a plant also varies depending on a plant species, and the amount of light received (Chen *et al*., 2016). In previous studies, the activity of superoxide dismutase SOD and catalase (CAT) enzymes were higher in sun-exposed *Changium smyrnioides* (Wang *et al*., 2017) and sun-exposed *Oryza sativa* (Gu *et al*., 2017). In *Dianthus caryophyllus* (Manivannan *et al*., 2017), and *Camptotheca acuminuta* (Yu *et al*., 2016), the antioxidant enzymes activity were higher under blue LED light. Variation in the antioxidant enzymes activities may be due to the level of stress encountered (acute or slow). Due to these differences, it is hypothesised in this study that "if the activity of antioxidant enzymes of a plant is low, then quercetin biosynthesis may increase".

1.2 Problem statement

In plants, genetic make-up, mutation and environmental factors are the major characteristics that contribute to variation in response to light. The above characteristics make it difficult to come up with a general conclusion on how plants respond to light. *Labisia pumila* and *Ginkgo biloba* can accumulate a higher amount of quercetin under high light intensity while *Berberis microphylla* and *Lithocarpus litseifolius* require a semi-shaded environment for their maximum quercetin accumulation. Due to these variations, there is a need to know at which level of light intensity will the selected plants accumulate high amount of quercetin.

Some authors hypothesised that lower light intensity favours the accumulation of monohydroxy B-ring flavonoid while high light intensity favours the accumulation of dihydroxy B-ring flavonoid. Quercetin falls under the latter group, but in some plants, it is not produced under high light intensity. In addition, the selected plants are abandoned, common tropical plants. There is no available study on the photoprotective role of quercetin to the plants. Moreover, there is no available information on the activity of antioxidant enzymes and oxidative stress of

the plants. This makes it necessary to test the hypothesis on the selected plants, and determine whether high light intensity will favour the accumulation of quercetin.

Some secondary metabolites like salicylic acid can affect plant photosynthesis, where a high concentration of the acid can decrease photosynthesis. A previous hypothesis on how quercetin may decrease plant photosynthesis by inhibiting the activity of ATPase and electron transport rate indicates that flavonoids can also affect photosynthesis. Quercetin can inhibit electron transport chain of *Croton ciliatoglandulifer*. This provides the need to know whether quercetin accumulation can affect the plants photosynthesis.

When the light intensity is high, plants tend to accumulate high amount of secondary metabolites. This makes it necessary to know whether the production of high amount of secondary metabolites under high light intensity is due to high photosynthesis or due to the production of photoprotective secondary metabolites for protection against the excess light.

Lastly, the selected plants have different genetic makeup and grow in different light conditions (sun-exposed, semi-shaded and shaded environments). This provides the need to know how varying light intensity can influence the photosynthesis, pigment, and total flavonoid contents of the plants growing in their natural environment.

1.3 Aim and objectives of the study

This research aimed at identifying the photoprotective role of quercetin to selected light-adapted and shade-tolerant plant species. This was achieved by following specific objectives of the study, which are:

i. To analyse how varying light intensity affects some gas exchange characteristics, pigment and production of total flavonoid content (TFC) of sun-exposed, semi-shaded and shaded plant species.
ii. To evaluate the photoprotective role of quercetin to the study plants.
iii. To correlate how the production of quercetin can affect the photosynthesis of the selected light adapted and shade tolerant plants

1.4 Scope of the study

This research focused on identifying the photoprotective role of quercetin to selected light-adapted and shade-tolerant plant species. *Mikania micrantha*, *Clidemia hirta* and *Tetracera sarmentosa* were selected for the study because the research is aimed at identifying the photoprotective role of quercetin to plants growing in their natural environment. Besides, the studied plants were found growing in sun-exposed, semi-shaded, and shaded regions of the mountain. Other reasons that justify selection of the plants include their medicinal application and their ability to grow in about 60 % of the forest. The initial step was achieved by obtaining approval from Perbadanan Taman Negara Johor (PTNJ). The plants were then selected and identified. The gas exchange was measured using the LI-6400 portable photosynthesis system. The total photosynthetic pigment contents, anthocyanin, total flavonoid, antioxidant enzymes, malondialdehyde, total soluble sugar, and soluble protein content of the plants were determined spectrophotometrically. Quercetin was quantified using high-performance liquid chromatography (HPLC). Finally, the results obtained were analysed statistically using SPSS statistical software.

1.5 Significance of the study

This research points out the benefit of producing quercetin to the plants. The findings of the study provide knowledge on the photoprotective role of quercetin to plants growing in their natural environment. This is necessary because most of the informations available on the photoprotective role of flavonoids were performed under a controlled environment. It also provides knowledge on the necessary measures required for improving the physiology of the studied plants by suggesting the best light condition for the selected plant's maximum photosynthesis. Moreover, it helps in providing information on how the studied plants survive in a constantly changing environment (that is how shaded plants tolerate lower light intensity and how sun exposed plants adapt to high light intensity), in addition to the knowledge on how plants use secondary metabolites instead of antioxidant enzymes for protection from reactive oxygen species generated due to excess light. The research provides answers to the tested hypothesis (how the activity of antioxidant enzymes is

related to quercetin biosynthesis, how light intensity affects monohydroxy B-ring and dihydroxy B-ring flavonoids, and how quercetin accumulation may affect photosynthesis). The findings identified the plants with high CO_2 absorption capacity which can be used for bioremediation and rehabilitation applications. Furthermore, the findings can be used by phytochemists when considering massive extraction of flavonoids.

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